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Peanut contamination by *Aspergillus flavus* and aflatoxin B1 in granaries of villages and markets of Mali, West Africa

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Abstract

Peanut (*Arachis hypogoea*) is an important crop in Mali, West Africa. However, one of the main problems related to peanut production and commercialization is the detection of high levels of aflatoxin B1 in peanut-derived products for consumption. The objectives of this study were 1) to determine the rate of progress of *Aspergillus flavus* and aflatoxin B1 in granaries located in 26 villages and also in the granaries located in the corresponding 26 markets in Mali and 2) to correlate the progress of the *A. flavus* and aflatoxin B1 with environmental variables outside the peanut granaries. The results showed that peanuts stored and consumed in rural areas are highly contaminated by *A. flavus* and aflatoxin B1, with average rates of aflatoxin B1 significantly above the accepted international standards especially between the months of June and December. There was a moderate relationship ($r^2 = 0.58$) between the aflatoxin B1 levels for two granary types, located at the villages and the corresponding granaries in the markets. The analysis of the aflatoxin B1 levels in the villages and its relation with the weather conditions outside the granaries revealed that for one village (Tiele), high relative air humidity was a contributing factor in the development of *A. flavus* and levels of the aflatoxin B1 in the granary. The results indicated that maximum aflatoxin B1 production occurs in presence of warm temperatures and relative humidity above 80%.

Key words: Groundnut, post-harvest, environmental variables, climate, weather, temperature, relative humidity, food security, health.

Introduction

Peanut is an important crop in West Africa, with an area planted in the region greater than 5 million ha during the period 2005 to 2007 [1]. Peanut is used and processed in diverse forms as grain, butter, oil, flour, dried leaves or stems. Therefore, it is a very important food source for more than 95% of households as well as a source for animal feed. The peanut crop also constitutes an essential source of revenue for the rural population and for the entire country of Mali. Peanut yields are usually low in Mali, compared to other production regions, with a national average, based on data reported from 2000 to 2005, of approximately 780 kg ha^{-1} [1].

Aflatoxin contamination of peanut is a serious quality problem in West Africa [2]. The main constraint in peanut production in Mali is the presence of an increased contamination of aflatoxin B1 in the peanut grain during traditional storage prior to marketing. Aflatoxins are toxic, carcinogenic, teratogenic and immunosuppressive substances produced when toxigenic strains of the fungi *A. flavus* Link. Ex Fries and *Aspergillus parasiticus* Speare grow on peanuts as well as many other agricultural commodities [3-6]. Infection of peanut by *Aspergillus* occurs under both pre- and post-harvest conditions. Thus, the contamination process is frequently broken down into two phases with the first phase occurring on the developing crop, i.e. pre-harvest, and the second phase affecting the crop after maturation during harvest and storage, i.e. post-harvest. Rain and temperature influence the phases differently with dry and hot conditions favoring the first phase, while warm and wet conditions favoring the second phase. Contamination varies with climate both temporally and spatially [7]. Mali has favorable climatic conditions for *Aspergillus* development; the country lies within the intertropical zone and has a hot, dry climate, with a conspicuous rainy season.

Pre-harvest infection by *Aspergillus* and consequent aflatoxin B1 concentration are more important in the semi-arid tropics, especially when drought occurs during the last 20-40 days of the season [8]. Other authors found that pre-harvest contamination requires a drought period of 30-50 days and a mean soil temperature in the podding zone of 29-31°C [9,10]. Similar results were also reported for peanut grown in Niger [11]. Climate and local weather have a strong impact on contamination [12], in part, by direct effects on the causative fungi [7]. Climate and local weather affect the rate of development of aflatoxin-producing fungi and alters the fungal community's structure. These factors also change the predisposition of hosts to contamination by altering crop development and insects' infestations that create wounds on which aflatoxin-producers fungi proliferate. Aflatoxin contamination is prevalent both in warm humid climates and in irrigated hot deserts. In temperate regions, contamination may be severe during drought [7].

For peanuts that are stored in enclosed facilities such as granaries, the local weather conditions as well as the environmental conditions in the storage facilities, especially temperature and relative humidity, are important for the growth of *A. flavus* and consequently present a potential risk for aflatoxin production [13]. The thermal conditions for *A. flavus* growth range from 10-12°C to 42-43°C, with an optimum in the 32 to 33°C range [14]. Fungal growth in storage facilities is favored by relative humidity above 83-85% [15]. Since these suitable conditions for growth and toxin production occur in many areas of Africa, the aflatoxin B1 contamination is usually a problem across the continent [16].

However, aflatoxin contamination of peanuts, which occurs both before and after harvest, can be effectively managed in order to produce shelled peanuts that meet strict regulatory guidelines, ensuring a safe food supply. This management primarily involves techniques that remove highly contaminated kernels from the majority that are not contaminated. However, these removal steps are costly, in terms of both processing and unavoidable loss of non-contaminated kernels. It is, therefore, highly preferable to take steps to prevent contamination if at all possible. Such steps include control of kernel moisture both before and after harvest. Prevention of pre-harvest aflatoxin contamination can also be achieved by harvesting peanuts before contamination occurs, where possible. However, this early harvesting can result in reduced yield and thus a reduced income for the farmer. Biological control technology has recently been commercialized which prevents much of the contamination that would otherwise occur [17, 18]. Best practices to achieve the lowest possible levels of contamination are to combine all possible management and prevention strategies to ensure and maintain a safe supply of peanuts [17]. Several technologies that can reduce risks of aflatoxin contamination have already been developed and tested in Mali [19]. These technologies include genetic resistance and integrated crop management practices, agronomic practices, biological control and biotechnological interventions. A number of agronomic practices that minimize risk of pre-harvest infection by *A. flavus* were tested in two major groundnut growing regions (Kolokani and Kayes) in Mali. The application of lime and farmyard manure significantly reduced aflatoxin contamination, especially in a susceptible cultivar. Lime application alone reduced aflatoxin by 79% and the application of farmyard manure reduced the aflatoxin content by 74%. Several harvesting and drying techniques, such as avoiding damage to pods, harvesting at right maturity, and proper drying of pods were also studied in Mali. The aflatoxin reduction under these practices varied from 69 to 88% at Kolokani, and 63 to 84% at Kayes [19].

Since there is an association between consumption of highly aflatoxin contaminated stocks of peanuts and impact on human health, mainly related to the prevailing types of cancers and other diseases [6, 20], it is extremely important to investigate the effects of environmental variables that affect the *A. flavus* growing conditions in the granaries. The objectives of this study were first, to determine the progression of *A. flavus* and aflatoxin B1 levels in granaries located in villages and markets of Mali, and second, to determine the correlation between the progression of *A. flavus* and aflatoxin B1 with environmental variables outside the peanut granaries.

Materials and Methods

During the period 1999/2000, 2000/2001 and 2001/2002 peanut samples were taken every three months from 26 granaries at villages and 26 granaries at the local markets of Mali, West Africa. Small weather station data loggers Hobos (Onset Computer Corporation) were installed in two granaries to monitor the temperature and air moisture. In February, April, June, August, October and December, peanut samples of one kg were collected from the previously mixed stock and then submitted to a laboratory for determination of *A. flavus* levels. For this purpose 20 peanut pods were vigorously shaken in a flask with sterilized water [21]; the resulting solution was placed on Petri dishes that contained a substratum consisting of boiled meat that was maintained for six days at room temperature (36°C). Three replicates were made from each sample. At the end of the six-day incubation procedure, the germinating *A. flavus* colonies were visually identified and counted.

The aflatoxin B1 was determined by the ELISA (enzyme linked immunosorbent assay) technique developed by ICRISAT [21]. For this, peanut samples of 100 g were taken from village and market stocks. An indirect determination of aflatoxin B1 was conducted by competitive binding.

A statistical analysis was conducted to test the differences in the level of weather variables for the locations with the highest and the lowest levels of aflatoxin B1. The Student's paired t-test and Freese test [22] were performed to evaluate the daily temperature and relative air humidity differences between the same months of different years. Lack of sufficient weather data was a limitation for most of the sites analyzed, therefore, the analysis focused on the sites with available weather information. In some cases data from nearby weather stations were used.

The original data for *A. flavus* and aflatoxin B1 had a high standard deviation for different years and months studied. Therefore, a data transformation was conducted to illustrate the relation between the *A. flavus* and aflatoxin B1 contamination of the granaries located in the villages and at the markets. The natural logarithms (ln) of the *A. flavus* and aflatoxin B1 levels were calculated to normalize the data for further regression analysis.

Results and Discussion

Climatic conditions in South Mali: The analysis of the weather records for 19 years in Bamako, Mali (latitude 12.63, longitude -7.98, elevation 200 m) revealed that the highest temperatures occur during April and the lowest temperatures during July and August. In addition, there is a conspicuous rainy season that starts in May and ends in October (Fig. 1). The analysis of the maximum relative humidity for the month of June indicated that the peanut area also had high relative humidity. In 1999, only three sites had a maximum relative humidity above 83.3% while in 2000 and 2001 there were five and six sites in this category, respectively (Fig. 2). During the three years that were studied, July and August consistently had a high maximum relative humidity in the peanut growing region.

Aspergillus flavus and aflatoxin B1 in the granaries of the villages: The analysis of the infestation in the 26 village granaries during the three years of the experiment showed a low level of *A. flavus* (spores g^{-1}) in February, and a consistent increase from February to August (Fig. 3). There was a plateau in the development of the fungus from August to December. However,

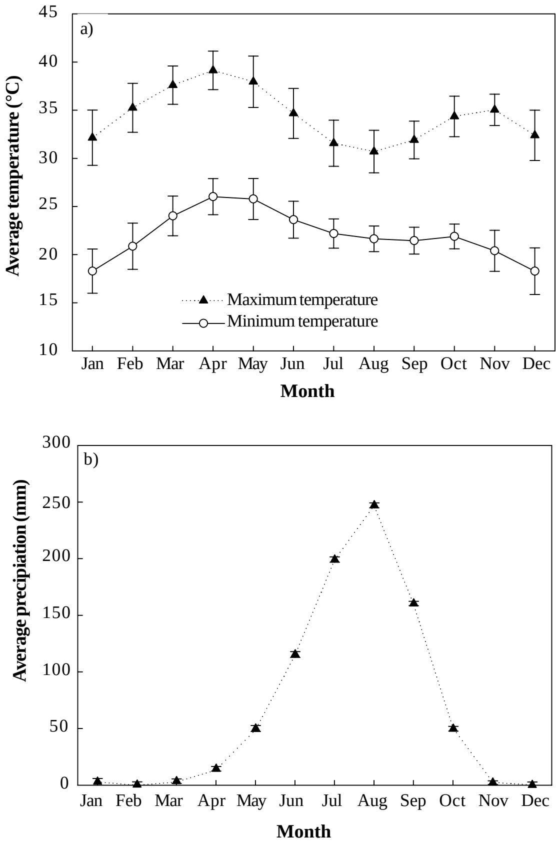

Figure 1. Average maximum and minimum air temperature (a) and average precipitation (b) for Bamako, Mali, for 19 years of weather records from 1981 to 1999.

the standard deviation was high during these months, indicating a significant variation between the granaries of the different villages. The correlation coefficient (r) between the average number of *A. flavus* of spores in the village granaries and the aflatoxin B1 amount was 0.85, 0.83, and 0.85 in 1999, 2000 and 2001, respectively. This indicated that the infection with *A. flavus* was related to the presence of aflatoxin B1 in the granaries for the three years of the experiment. The year 2001 had the highest level of *A. flavus* and the highest standard deviation.

Similarly to *A. flavus*, the aflatoxin B1 levels were low in February for all granaries of the individual villages. However, there was a consistent increase from April to December, reaching levels above the international standard as early as June and rising thereafter. It is important to mention that the maximum allowable level in the United States is 20 ppb and the European Union has an allowable level of 4 ppb total aflatoxins and under 2 ppb aflatoxin B1 [23].

There was a considerable variation in the levels of aflatoxin B1 from June to December (Fig. 3). The granaries with the highest average aflatoxin B1 were located in the villages of Diaka, Tiele, N'tonsoni and Konko, while the lowest values were found in the granaries located at Lofigué, Lofiné, Loulouni and Bogi (Fig. 4). The average aflatoxins B1 levels (expressed as ln), for the three years and the 26 villages are represented in a map (Fig. 5). The highest levels of aflatoxin B1 were found in 2001.

A. flavus and aflatoxin B1 at market granaries: The *A. flavus* levels in granaries located at markets revealed differences between years, with the highest level recorded in 2001 (Fig. 6). This could be due to the favorable environmental conditions, but also to poor sanitation of the granaries and poor quality of the peanuts prior to storage. The average level of aflatoxin B1 detected from June to December in the market granaries was significantly higher than the accepted international standard, and there was a high variation between the granaries. This illustrated the severe problem with aflatoxin B1 in the market granaries. The granaries with the highest average levels of aflatoxin were found at the markets in Fouya, N'tonsoni, Banlandou and Tiele, while those with the lowest average level were found at the markets in Soukoumba, Lofigué and Diomatene (Fig. 7).

Relation between Aspergillus flavus and aflatoxin B1 levels in the village and market granaries: The original data for *A. flavus* infection levels and aflatoxin B1 had a high standard deviation for the different years and months that were studied. The regression analysis showed a low coefficient of determination between *A. flavus* at the village and market granaries and between the aflatoxin B1 levels in the granaries at the villages and the corresponding markets. Data were transformed to natural logarithms (ln) to more clearly illustrate the relation between the *A. flavus* and aflatoxin B1 at the two types of granaries. There was a moderate relation between the number of *A. flavus* found at the village granaries and the market granaries ($r^2 = 0.39$) (Fig. 8a).

The coefficient of determination (r^2) was 0.58 between the aflatoxin B1 levels in granaries at the villages and the corresponding granaries in the markets (Fig. 8b), showing that there was a moderate relationship between the aflatoxin B1 levels for the two granary types. It was hypothesized that the weather conditions occurring during storage in the villages would be similar to the weather conditions during storage on the markets because both types of facilities are located in the same micro-region. However, it seems that other factors different from the weather conditions outside the granaries also played a role in the development of *A. flavus* and consequently on the levels of aflatoxin B1. Poor sanitation in the granaries before peanut storage and differences in ventilation between the granaries could also have an impact on the development of the fungus inside the facilities. However, our results showed that the weather conditions outside the granaries enhanced the development of *A. flavus*, especially during the rainy season. Further analysis was conducted to identify which particular weather conditions would favor the development of this fungus.

Analysis of weather variables for locations with contrasting levels of aflatoxin B1: Tiele had one of the highest average levels of aflatoxin B1 in the village granary in 2000 (1654 ppb) and relatively low values in 1999 (47 ppb) and 2001 (114 ppb). As the weather data for Tiele were not available, the data for Bamako Senou were used, located approximately 50 km from Tiele. A statistical analysis was conducted to determine whether there were significant differences in weather data for the three years. The analysis of the rate of aflatoxin B1 increase revealed that the greatest increase in 2000 occurred between June and August. Thus, the statistical analysis of daily weather data focused on the period between June and August for the three years.

The average monthly minimum temperature for June was 24.6°C, 22.7°C and 23.6°C in 1999, 2000 and 2001, respectively (Fig. 9a).

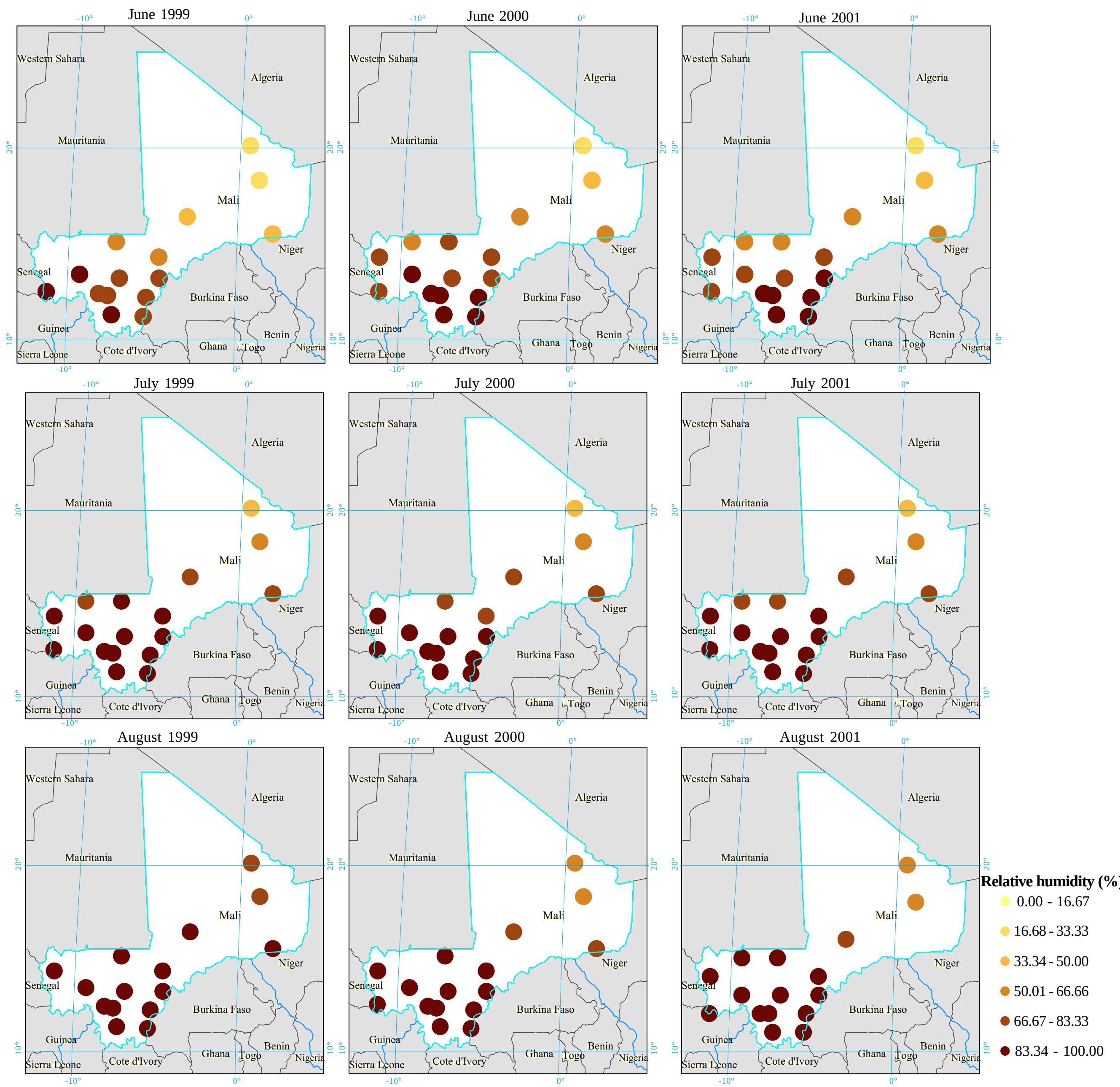

Figure 2. Average monthly maximum relative air humidity for June, July and August for 1999, 2000 and 2001.

Statistical differences were found for minimum temperature between June 1999 and June 2000 and also between June 2000 and June 2001 using Freese [22] and the t-test. The average maximum temperature for June 1999, June 2000 and June 2001 was 35.9, 33.5 and 34.0°C, respectively. Statistical differences were found for maximum temperature between June 1999 and June 2000 (Freese [22] and t-test). The average maximum temperature for August 1999, August 2000 and August 2001 was 28.3, 30.4 and 31.0°C, respectively (Fig. 9b). Significant differences were found for maximum temperature between August 1999 and August 2000 and between August 1999 and August 2001.

The average minimum relative humidity was 34.3% for June 1999 and 47% for June 2000 and 2001 (Fig. 10a). Statistical differences were found for minimum relative humidity between June 1999, June 2000 and June 2001. The average maximum relative humidity for June 1999, June 2000, August 2000 and August 2001 was 79%, 91%, 99% and 98%, respectively (Fig. 10b). Statistical differences were found for maximum relative humidity between June 1999 and June 2000 and also between August 1999 and August 2000 (Freese [22] and t-test).

At Tiele, the favorable temperatures (33.5°C maximum temperature, 22.6°C minimum temperature) and the high relative humidity during June 2000 (91.1% maximum humidity) stimulated the development of the *A. flavus* and consequently increased the aflatoxin B1 levels in the granaries in the village. Therefore, it seems evident that weather conditions should be considered for proper granaries' management. Actions should be taken whenever favorable conditions for *A. flavus* infection are forecasted or met, in order to avoid the increase in aflatoxin B1. As a practical management approach for the granaries, the humidity inside the

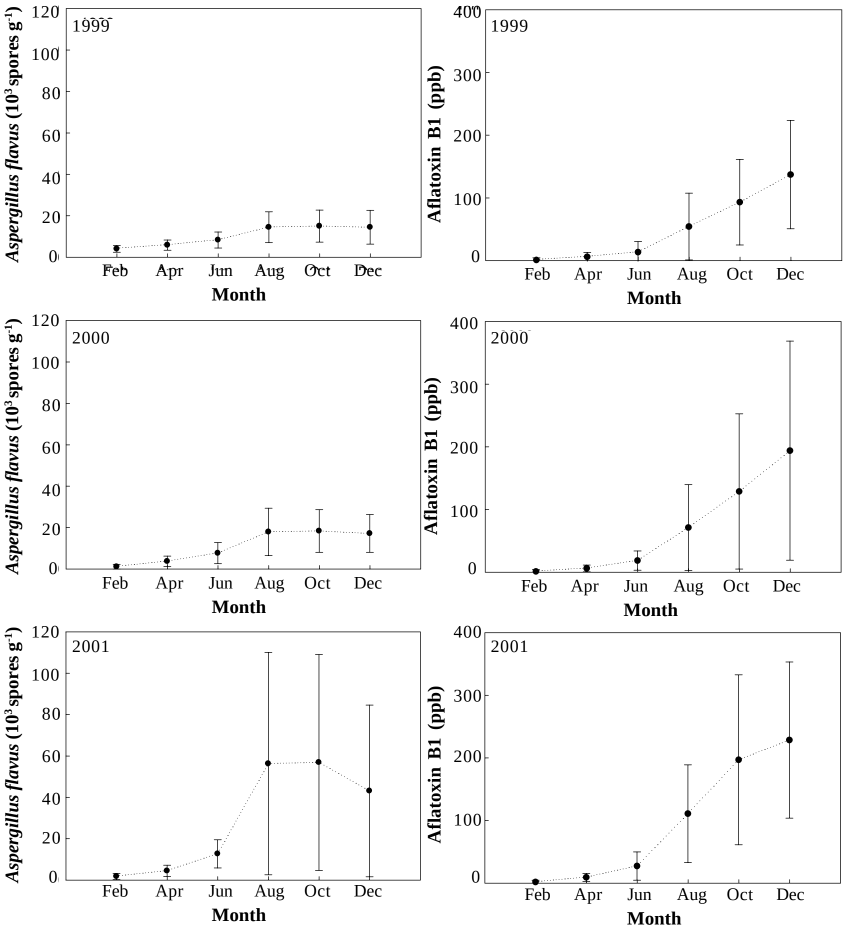

Figure 3. Average and standard deviation for levels of *Aspergillus flavus* and aflatoxin B1 for 26 granaries located in different villages in Mali for 1999, 2000 and 2001.

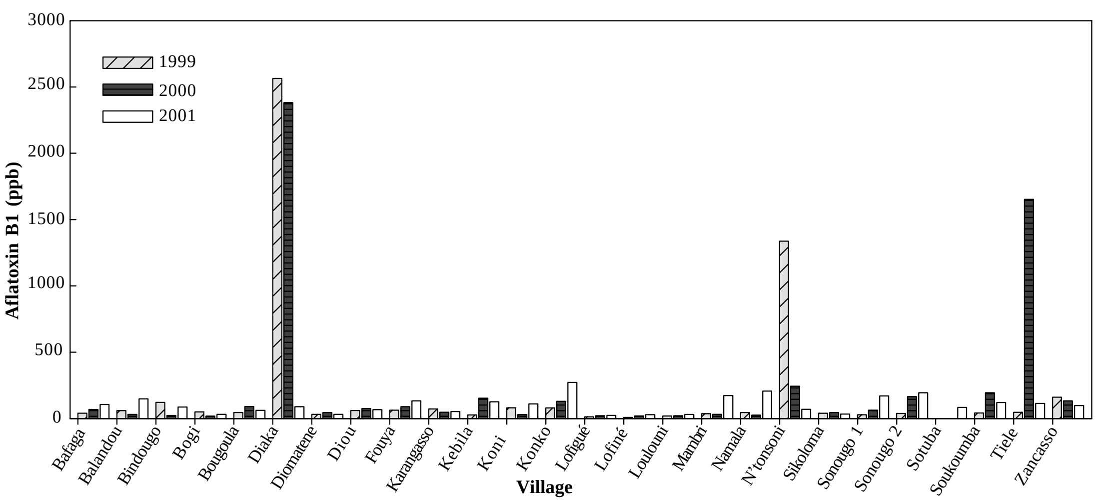

Figure 4. Average aflatoxin B1 levels for 26 granaries located in different villages in Mali for 1999, 2000 and 2001.

granaries could be reduced by promoting proper ventilation, because peanuts stored with aeration generally cool faster than those stored without aeration [24]. Aflatoxin detected in the initial peanut samples of the Southeastern USA was significantly reduced by aeration in the storages.

The results from our study indicated that Mali has conditions for A. *flavus* growth in the granaries and consequently for aflatoxin B1 contamination, with the south-west region of Mali being the most affected area, which is due in part to the high relative humidity. Warm temperatures and relative humidity above 80% promoted the development of A. *flavus* in the granaries. Similar results have been found in other regions where maximum toxin production occurred between 25 and 27°C and fungal growth in storage facilities was favored by relative humidity above 85% [16].

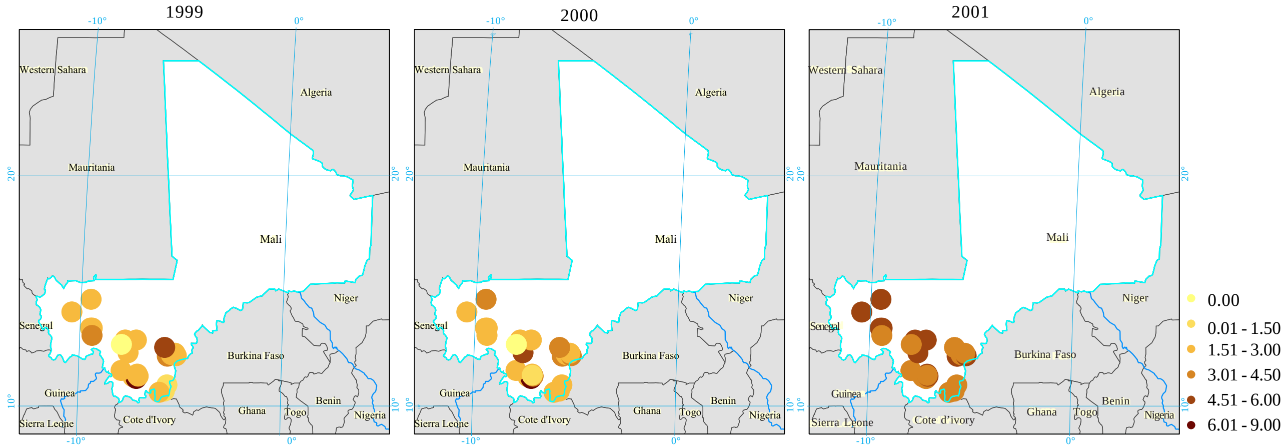

Figure 5. Average aflatoxin B1 levels (expressed as ln) for 26 granaries located in different villages in Mali for 1999, 2000 and 2001.

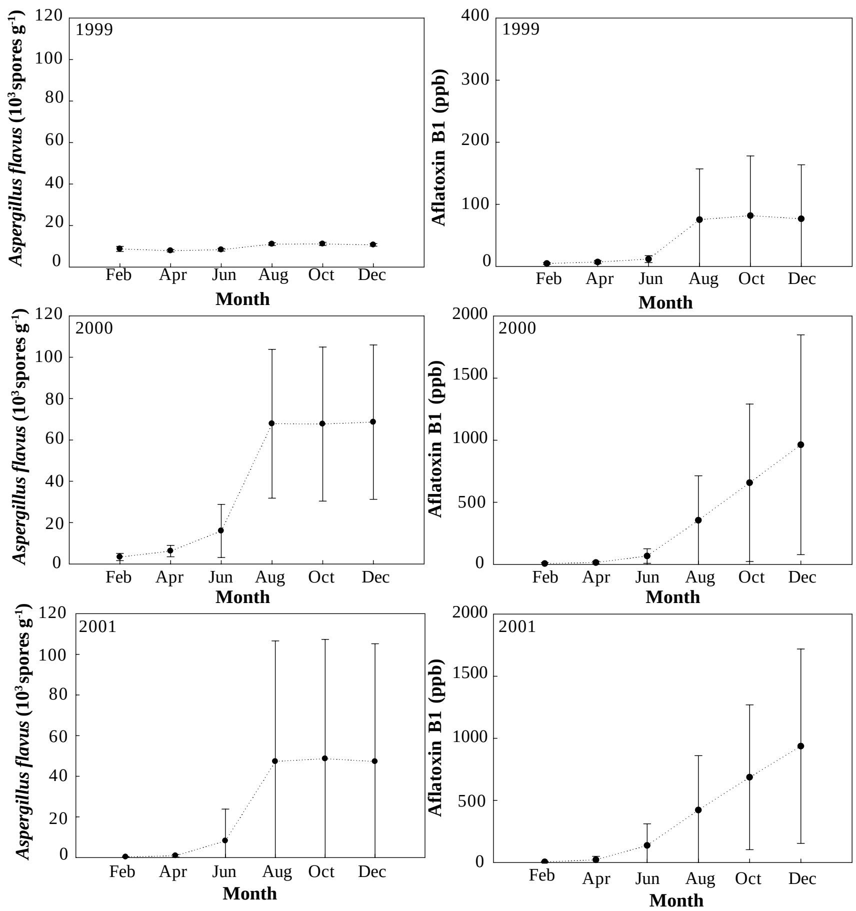

Figure 6. Average and standard deviation levels of *Aspergillus flavus* and aflatoxin B1 for 26 market granaries in Mali for 1999, 2000 and 2001.

Studies conducted in Brazil showed that *A. flavus* growth was promoted by an increase in relative humidity accompanied by temperatures in the range of 25-30°C in infected nuts [25]. Similarly, studies conducted in Pakistan revealed that the higher the humidity level, the faster the growth of *A. flavus* growth and the higher the toxin production [26]. A pattern whereby the percentage of contaminated peanut samples on farmers' granaries declined with decreasing precipitation across Western Kenya has been reported [27].

In 2000, a high level of aflatoxin B1 was observed at Tiele in both the village and market granaries. In the market granaries the average aflatoxin B1 level was 413 ppb, 977 ppb and 143 ppb in 1999, 2000 and 2001, respectively. The same weather data were used for analysis of the markets and villages granaries at this location (Tiele). The month of June was critical for development of *A. flavus* and aflatoxin B1 in Mali, since a significant increase in the rate of the peanut contamination in many granaries was observed during this month. Other sites with high aflatoxin B1

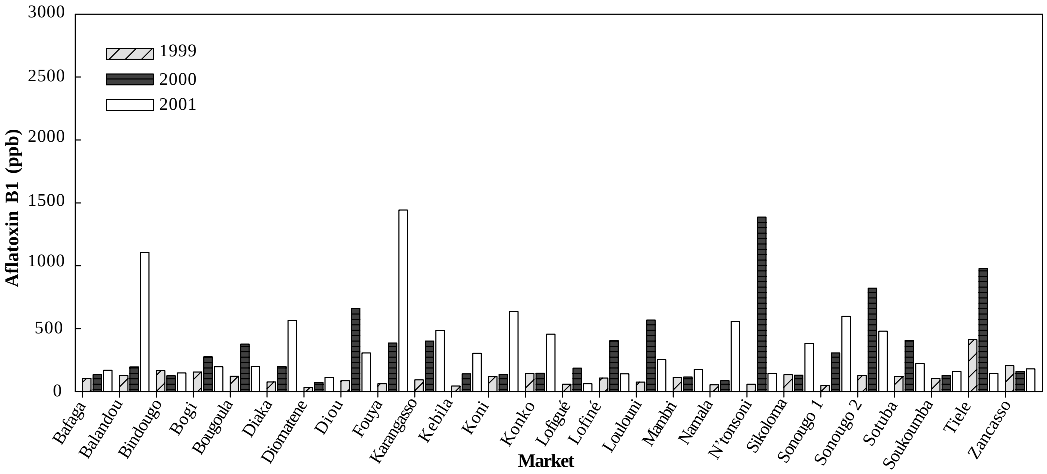

Figure 7. Average levels of aflatoxin B1 for 26 granaries located in different markets in Mali for 1999, 2000 and 2001.

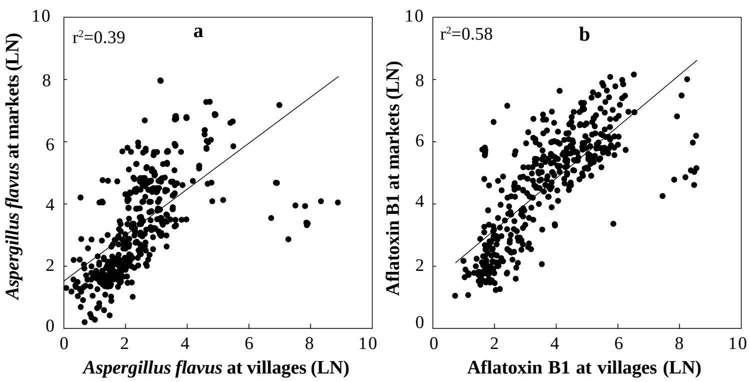

Figure 8. Relation between *Aspergillus flavus* levels at the 26 villages and the *Aspergillus flavus* levels in the corresponding 26 markets for 1999, 2000 and 2001 (a); relation between aflatoxin B1 levels at the 26 villages and the aflatoxin B1 levels in the corresponding 26 markets for 1999, 2000 and 2001 (b).

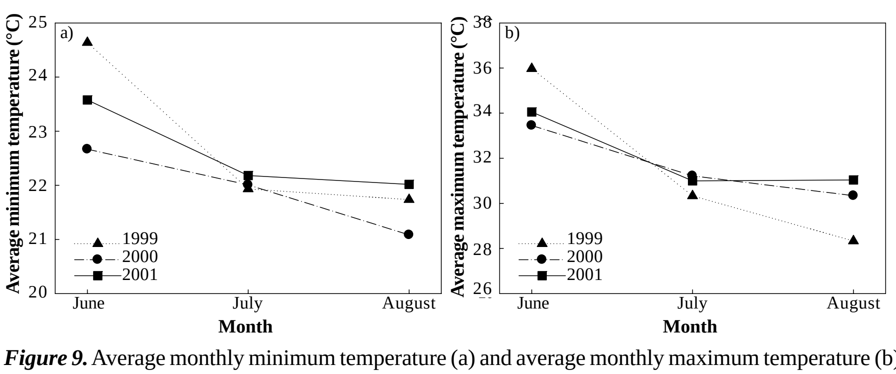

Figure 9. Average monthly minimum temperature (a) and average monthly maximum temperature (b) for the months of June, July and August recorded at Bamako Senou (near Tiele).

levels at the village granaries included Diaka and Ntonsoni. Unfortunately, these sites had only limited weather data, and, therefore, no statistical analysis was conducted.

Lofigué, Lofiné and Loulouni had the lowest aflatoxin B1 in the villages. These sites are located in the southern part of Mali, near the border with Burkina Faso. For some villages, like Bindougou, Lofigué and Lofiné, the only weather data available were temperature and relative humidity obtained every 90 min with the Hobos located inside the granaries (Figs 11-13). Unfortunately, technical problems with the Hobos prevented collection of a complete data set for analysis. However, based on partial data collected, it was evident that there was an increase in the relative humidity from June to August inside the granaries at Lofigué (Fig. 12) and Lofiné (Fig. 13). The maximum relative humidity

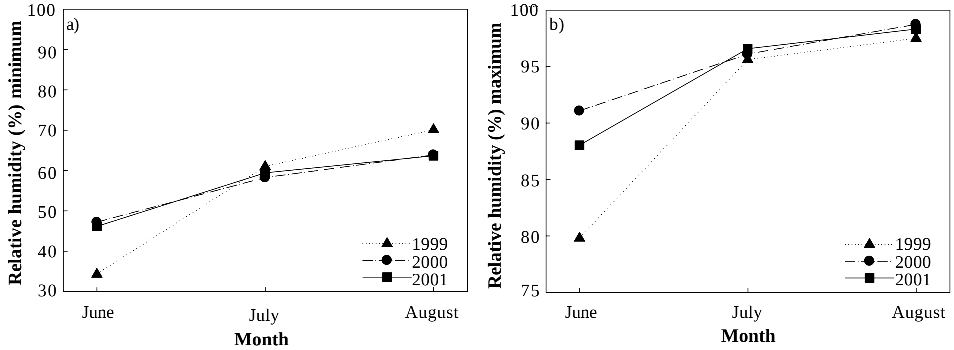

Figure 10. Average monthly minimum relative air humidity (a) and average monthly maximum relative air humidity (b) for the months of June, July, and August at Bamako Senou (near Tiele).

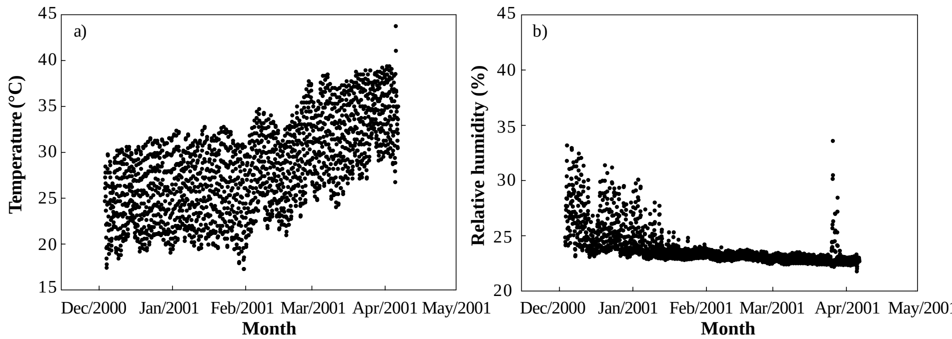

Figure 11. Temperature (a) and relative humidity (b) from December 2000 to April 2001 for the peanut granary located at Bindougou.

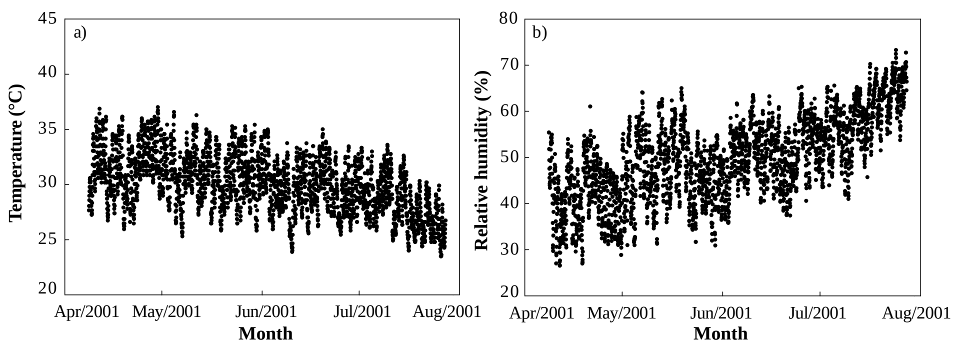

Figure 12. Temperature (a) and relative humidity (b) from April 2001 to July 2001 for the peanut granary located at Lofigué.

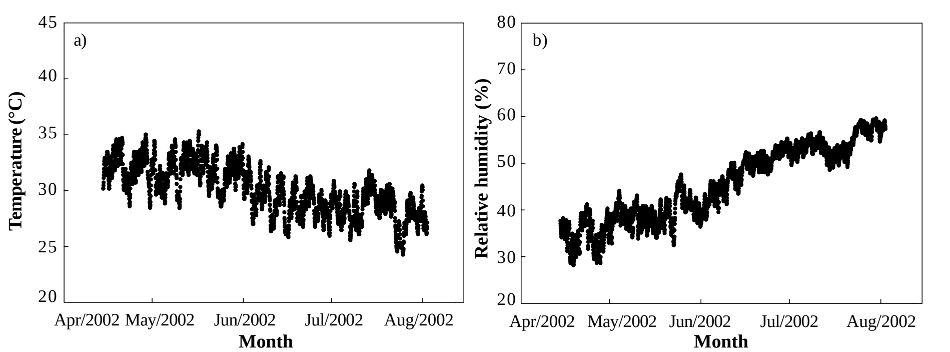

Figure 13. Temperature (a) and relative humidity (b) from April 2002 to August 2002 for the peanut granary located at Lofiné.

only reached 60 and 70% in August for Lofiné and Lofigué, respectively, indicating that an extreme problem with aflatoxin B1 would not be expected for these two locations.

Summary and Conclusions

In this study *A. flavus* development in peanut stocks and the heavy contamination of aflatoxin B1 in village and market granaries of Mali were analyzed. The results showed that weather conditions played an important role in the development of *A. flavus*. Environmental conditions (temperature and relative humidity) favorable for *A. flavus* development and aflatoxin contamination were identified. The results indicate that the weather conditions outside the granaries enhanced the development of *A. flavus*, mainly during the months of June, July and August; warm temperatures and relative humidity above 80% promoted the fungal growth in storage facilities. However, the limited weather data that were collected at specific granaries, made it difficult to conduct a thorough statistical analysis. Nevertheless, the results and discussion presented in this study could help enhance management of peanut granaries. Agricultural extension programs in this region should focus on the development of proper storage facilities with sufficient ventilation that reduces the high relative humidity levels required for *A. flavus* development and contamination of peanuts by aflatoxin. A good sanitation program could be developed for local granaries and other facilities to eliminate or reduce the carry-over inoculum from prior years' peanut storage.

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